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TECHNICAL MANUSCRIPT 388

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PRODUCTION BY DESULFOVIBRIO

Warren P. Iverson

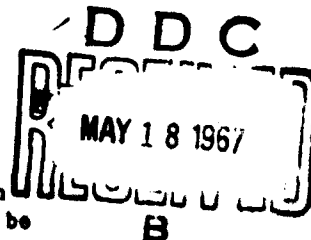
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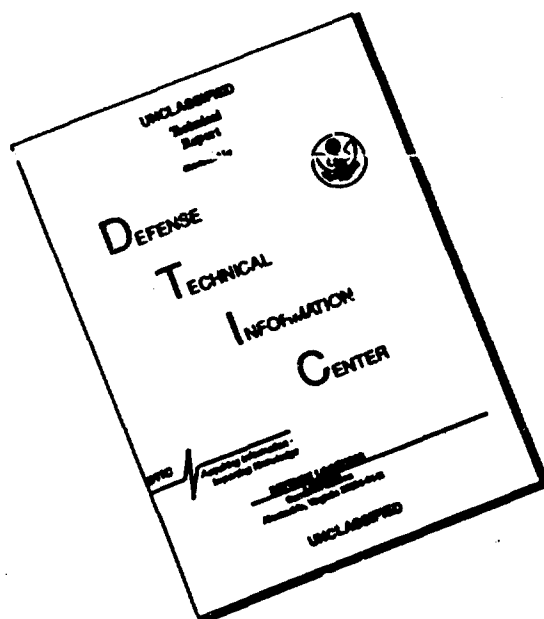
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Warren P. Iverson

Special Operations Division  
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY

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## DISULFUR MONOXIDE: PRODUCTION BY DESULFOVIBRIO

### ABSTRACT

Desulfovibrio desulfuricans growing on agar surfaces produces a gas that appears to be identical to "Schenk's sulfur monoxide," later identified as disulfur monoxide. The gas stimulates surface growth of Desulfovibrio on an agar medium and is used by the cells as an electron donor for the reduction of benzyl viologen.

Previous investigations with Desulfovibrio desulfuricans (Mid-Continent strain A) had indicated the presence of a volatile substance or gas that was produced by this organism growing on plates of trypticase soy broth\* plus 2% agar.<sup>1</sup> This gas was characterized by its ability to stimulate growth of the same organism on agar plates of API\*\* medium<sup>2</sup> where growth was ordinarily very poor and also by its ability to be used by the organism as an electron donor for the reduction of benzyl viologen (BV). Violet (reduced) areas of the dye were produced in agar [2% agar plus 0.01 M tris(hydroxymethyl)aminomethane - HCl buffer, pH 7.0 ± 1, and 0.01% BV] around heavy concentrations of Desulfovibrio cells when petri plates of the agar were placed in contact with this gas in a helium or nitrogen atmosphere.

Attempts to identify this gas by gas chromatography were unsuccessful. When the volatile material was flushed with helium from a Brewer jar containing inoculated trypticase soy agar plates through a series of dry glass traps (cooled with NaCl-ice, methanol - dry ice, and liquid nitrogen successively), the gas, as indicated by the two biological properties described above, could be concentrated in the liquid nitrogen trap. An odor resembling that of H<sub>2</sub>S was detected in the liquid nitrogen trap as well as from the outlet tube from this trap, which was immersed in water. The water produced blackening of lead acetate paper. The odor of H<sub>2</sub>S could be detected, however, only infrequently from a Brewer jar containing inoculated plates of trypticase soy broth plus agar. A sample of the gas from the trap in liquid nitrogen was analyzed in the mass spectrometer but no sulfur compounds could be detected. Substances with mass peaks of 44, 28, and 16 corresponding to CO<sub>2</sub>, N<sub>2</sub> or CO<sup>+</sup>, and CH<sub>4</sub> were detected. CO<sub>2</sub> and CH<sub>4</sub> have previously been reported as metabolic products of Desulfovibrio.<sup>3,4</sup> Because the trap, cooled with liquid nitrogen, was evacuated to remove helium before analysis, the compound might have been removed. In an attempt to isolate the sulfide compound,

\* Baltimore Biological Laboratories.

\*\* American Petroleum Institute.

the biologically produced gas was passed (using helium as a carrier) through a series of three lead acetate traps (5% solution w/v) and then to the atmosphere by bubbling through water. No darkening in any of the three traps was noticed, but a strip of lead acetate paper placed in the water exposed to the air darkened in a minute or less. An odor resembling that of  $H_2S$  was also present. The gas appeared to form a sulfide only when in contact with oxygen.

A small amount of black precipitate was obtained in a week by placing a lead acetate solution (5% w/v) in a petri plate on top of 12 inoculated plates of trypticase soy broth plus 4 ml per liter sodium lactate plus agar in a Brewer jar with an atmosphere of hydrogen with traces of oxygen present. The precipitate was collected and the gas resulting from the addition of concentrated  $H_2SO_4$  to the precipitate in nitrogen atmosphere stimulated growth on API agar (growth and blackening in 17 hours) and acted as a hydrogen donor for BV reduction.

Another sample of the precipitate was similarly obtained and the gas was analyzed in the mass spectrometer. Mass peaks corresponding to  $H_2S^-$ ,  $SO_2^-$ , and  $SO^-$  were found.

$H_2S$  had no stimulatory effect on growth and no hydrogen donor ability.  $SO_2$  similarly had no effect in stimulating growth on API agar, but very slight reduction of BV by Desulfovibrio cells did occur. "Sulfur monoxide," now established as  $S_2O$  by Meschi and Myers,<sup>5</sup> was prepared according to the method of Schenk<sup>6</sup> by passing an electrical discharge (about 5 kv) through  $SO_2$  (generated by adding concentrated  $H_2SO_4$  to anhydrous reagent grade  $Na_2SO_3$ ) in a straight glass tube (74 cm long; 2.5 cm I.D.) with platinum electrodes sealed at each end at low pressure (2 to 3 mm). The  $S_2O$  was flushed with helium into an evacuated Brewer jar with inoculated plates of API agar and BV agar. Sufficient  $S_2O$  was produced after repeating the procedure seven or eight times so that growth was visible on the API medium within 17 hours, as well as evidence of reduction of BV.

The infrared (IR) spectra of  $SO_2$  before and after electrical discharge are indicated in Figure 1, A and B. The absorption peaks for  $SO_2$  at 1,155  $cm^{-1}$  and 1,125  $cm^{-1}$  were replaced by a single peak at 1,135  $cm^{-1}$ . Peaks similar to those in Figure 1 B were also obtained by passing an electrical discharge through  $SO_2$  in S vapor, using the apparatus described by Jones.<sup>7</sup> The gas appears to be stable in a dry glass trap, as evidenced by the appearance of the 1,135  $cm^{-1}$  peak, for at least 24 hours. This stability is in agreement with the finding of Cordes and Schenk.<sup>8</sup>



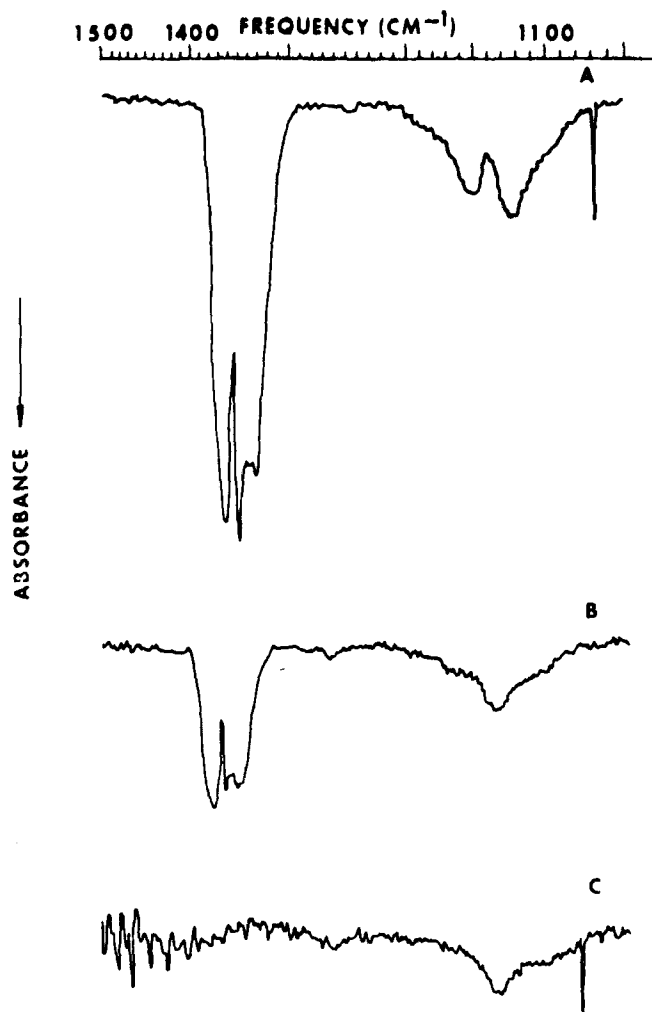


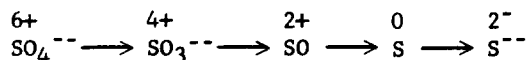
Figure 1. Infrared Absorption Bands. A. IR absorption bands of SO<sub>2</sub>; path length 10 cm; NaCl windows. B. IR absorption bands of SO<sub>2</sub> after passage of electrical discharge; path length 10 cm; NaCl windows. C. IR absorption band of gas produced by *Desulfovibrio* collected in liquid N<sub>2</sub> trap; path length 10 cm; NaCl windows.

The IR spectrum of the gas produced by Desulfovibrio and collected in the liquid nitrogen-cooled trap is shown in Figure 1 C. A peak at  $1,135\text{ cm}^{-1}$ , identical to the peak of the electrically discharged  $\text{SO}_2$ , is present. No bands for  $\text{H}_2\text{S}$  or  $\text{SO}_2$  were found.

The IR spectra reported by Jones<sup>7</sup> for  $\text{S}_2\text{O}$  indicates bands at  $1,165\text{ cm}^{-1}$  and  $679\text{ cm}^{-1}$ . Blukis and Myers,<sup>9</sup> using frozen films, reported that the  $679\text{ cm}^{-1}$  band disappears and the band at  $1,165\text{ cm}^{-1}$  decreases in intensity and shifts to  $1,300\text{ cm}^{-1}$  after heating the film to  $280\text{ K}$ . On further examination it appears that the band found at  $1,135\text{ cm}^{-1}$  and the one reported by Myers at  $1,130\text{ cm}^{-1}$  are probably identical and are due to a decomposition product of  $\text{S}_2\text{O}$ , the band being caused by an S-O stretch.

Schenk<sup>10</sup> and Murthy<sup>11</sup> reported that  $\text{S}_2\text{O}_3^{--}$ ,  $\text{S}^{--}$ , S were produced by "SO" or  $\text{S}_2\text{O}$  (because the properties previously attributed to the presence of SO in the discharge product are shown to be due to  $\text{S}_2\text{O}$ ) when it reacted with alkaline solutions. It was of interest that (0.02%)  $\text{Na}_2\text{S}_2\text{O}_3$  incorporated in the API agar also stimulated growth and acted as an electron donor for the reduction of BV.

The possibility is suggested that the organisms may be producing SO as an intermediate in the reduction of sulfate from trypticase soy broth (7 to 8 mg  $\text{SO}_4^{--}$  as the barium salt per gram dry medium):



SO is quite unstable, decomposing within 3 seconds, and probably forms  $\text{S}_2\text{O}$  immediately according to the following reaction:  $3\text{SO} \longrightarrow \text{S}_2\text{O} + \text{SO}_2$ .<sup>6</sup>  $\text{SO}_2$ , however, was not detected in the traps during these observations.

A large number of extracellular, irregularly shaped particles was found among the cells of Desulfovibrio that were reducing BV on agar in the presence of the gas produced by growth of the organism on trypticase soy broth agar plates. Using the technique described by Skerman,<sup>12</sup> in which sulfur is extracted and recrystallized from pyridine, a large number of rhombic crystals were found among the cells after several hours, suggesting that the organisms might have been producing sulfur. It cannot be too strongly stressed that until the complete products of the reaction of the gas with the agar are known, it would be impossible to state whether sulfur is produced by the organisms or by a direct breakdown of  $\text{S}_2\text{O}$  with water.<sup>11</sup>

It was previously mentioned that the gas has an odor resembling that of  $\text{H}_2\text{S}$  in contact with air. Cordes and Schenk<sup>13</sup> also observed a similar odor from their "sulfur monoxide." Murthy<sup>11</sup> reported  $\text{S}^{--}$  as a reaction product with  $2\text{N NaOH}$ .

It thus appears that the gas  $S_2O$ , or its decomposition product, is produced by Desulfovibrio growing on agar plates and that it may be an intermediate product in the reduction of sulfate.

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